DESIGNED BETA HAIRPIN PEPTIDES AS MODELS FOR BIOLOGICAL METALLOPROTEINS

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DESIGNED BETA HAIRPIN PEPTIDES AS MODELS FOR BIOLOGICAL METALLOPROTEINS

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NOMENCLATURE

PSII	Photosystem II
CA	Carbonic Anhydrase
PCET	Proton-Coupled Electron Transfer
OEC	Oxygen-Evolving Complex
NMR	Nuclear Magnetic Resonance
CD	Circular Dichroism
UVRR	UV Resonance Raman Spectroscopy

SUMMARY

Understanding the chemistry of metalloproteins can lead to innovative solutions to major problems in fields such as energy conservation and drug development. In nature, there are numerous biological proteins that use metal cofactors as part of their function. For example, Photosystem II contains a manganese-core that assists with the oxygen evolution process. Without the metal cluster, the protein is unable to produce the final products of photosynthesis. Carbonic anhydrase is another biological protein that needs zinc to convert carbon dioxide into bicarbonate. The zinc plays a critical role in the interchangeable conversion of these two molecules. By understanding the interactions between the metal and the protein, it is possible to harness the chemistry of these biological systems for commercial purposes.

This study investigates the interactions between a biologically inspired designed peptide and metals. Biomimetic peptide models, also referred to as maquettes, can be designed and used to study specific portions of complex biological proteins. This study has designed a peptide that contains the appropriate amino acid residues to facilitate metal binding. By introducing various relevant metals to the peptide system, spectroscopic methods were used to determine if the metal is binding. The results showed that the metal did indeed bind to the synthesized peptide. This study will provide a better understanding of the influence of metals in biological systems, and how these characteristics may be applied for synthetic purposes.

CHAPTER 1

INTRODUCTION

Metalloproteins play critical roles in many biological systems, such as facilitating reactions to stabilizing proteins. Without certain metals many enzymes would not be able to function. Two prime examples of metalloproteins are Photosystem II (PSII) and carbonic anhydrase (CA). Photosystem II contains a manganese based oxygen-evolving complex that helps facilitate the redox reactions involved in oxygen evolution (2). Carbonic anhydrase utilizes a zinc metal core to convert carbon dioxide into bicarbonate (3). Both of these proteins serve crucial roles in their respective organisms, and without the metal core these proteins would lose their functionality.

By further investigating the properties of these metalloproteins, it is possible to utilize the protein's mechanism for commercial purposes. For example, by understanding the fine details of PSII, an artificial solar energy conversion system can be created. Although the general mechanism of both PSII and CA are understood, further research needs to be conducted on how the metal cofactors specifically influence the pathways of these enzymes.

Biomimetic peptide maquettes serve as modifiable and functional models through which more complex proteins can be recreated through scaffolds (9). These models can be used as theoretical platforms in which the interactions of higher order proteins can be isolated and studied. The beta-hairpin structure also lends insight on the thermodynamic stability of a protein. Previously, a beta-hairpin structure, Peptide A, was synthesized and exhibited proton coupled electron transfer (PCET) properties like that of Photosystem II (8). Using Peptide A as a model, mutagenesis can be performed on the peptide

environment to further investigate various aromatic and electrostatic effects on the PCET reaction.

The current peptide under investigation, Peptide P, is modeled to have four histidine residue sites that can serve as metal coordination sites. Unlike previously synthesized peptides, this peptide will allow the investigation of coordination between metals and amino acid residues of metalloproteins. A previous study conducted by Rufo *et al*. (2014) showed that a synthetic peptide (with designed binding sites similar to that of Peptide P) successfully binds to zinc, forms amyloid fibers, and even exhibits catalytic properties (7). The overall goal of this project is to determine if Peptide P, which emulates the environment in two major biological metalloproteins, will also bind to metals and potentially catalyze a reaction itself. By doing so, Peptide P can serve as a fundamental model to represent a biologically inspired energy conversion system.

CHAPTER 2

LITERATURE REVIEW

Metal cofactors can serve a crucial role for the structure and function of certain enzymes and proteins. Photosystem II and carbonic anhydrase are proteins that have a metal-amino acid complex that is crucial for the proteins to carry out their function (2, 3). By better understanding the chemical interactions between these metal-ligand complexes, it is possible to apply the properties of these metalloproteins for synthetic and commercial purposes.

Photosystem II

Photosystem II (PSII) is the protein responsible for oxygen evolution in photosynthesis (2). This process involves using energy from the sun to transform water into oxygen and proton equivalents. The oxygen-evolving complex (OEC) is comprised of manganese, calcium, and oxygen and plays an essential role in water oxidation (2). For this reaction to occur, multiple electrons must be transferred within Photosystem II (1). Electrons transfer occurs through a process known as proton-coupled electron transfer (PCET), and the manganese-calcium cluster (Mn_4CaO_5) of Photosystem II binds to water and catalyzes the oxidation of water to produce O_2 and p. Control of Proton and Electron Transfer in de Novo Designed, Biomimetic β Hairpins rotons equivalents, and utilizes aromatic residues (such as tyrosine and tryptophan) to shuttle the electrons in a particular pathway (5). This results in the production of radical aromatic amino acids that can transfer electrons of the reaction (1, 6). During oxygen-evolution, the manganese metal cluster changes its oxidation state over a period of five “S-states” (11). Without this metal cluster, Photosystem II is unable to oxidize water.

Carbonic Anhydrase

Carbonic anhydrase is another metalloprotein that hydrolyzes carbon dioxide to bicarbonate. This is a reversible reaction that also involves water and protons (3). There are three types of carbonic anhydrase proteins (α, β, and γ), and they are present in mammals, higher order plants, and some bacteria (3, 4). There is a zinc residue in the core of the protein that plays a crucial role in this reaction. The function of the zinc is to increase the nucleophilicity of the water (4). The reaction also involves a proton-electron shuttling mechanism (3).

Biomimetic Peptide Maquettes

One way to study complex proteins, such as these metalloproteins, is by creating artificial biomimetic peptide maquettes (7, 8, 9). These maquettes can be designed to have the same environment as the protein of interest in an isolated manner. Previously, a peptide (Peptide A) was designed to emulate the PCET environment of Photosystem II (8). A NMR spectrum of Peptide A showed that it was in the beta-hairpin conformation, confirming that the histidine and tyrosine residues are in the appropriate position to interact (Figure 2). Through mutagenesis, it is possible to change the sequence of this peptide so that different properties can be studied. The current peptide (Peptide P) is designed with the same structure as Peptide A, except four histidine residues have been introduced. These histidine residues serve as metal coordination sites, similar to that of Photosystem II and carbonic anhydrase. Rufo *et al*. designed a small seven residue peptide that had a sequence similar to carbonic anhydrase and Peptide P, and this small peptide also forms amyloid fibers and catalyzes a reaction just as carbonic anhydrase (7).

While Peptide A has revealed information about the electron transfer reactions in a PSII-inspired system, Peptide P will take this model a step further by revealing how the metal complex of PSII can steer the electronic interactions that occur within the protein system. Figure 1 shows the sequence and structure of both Peptide A and P.

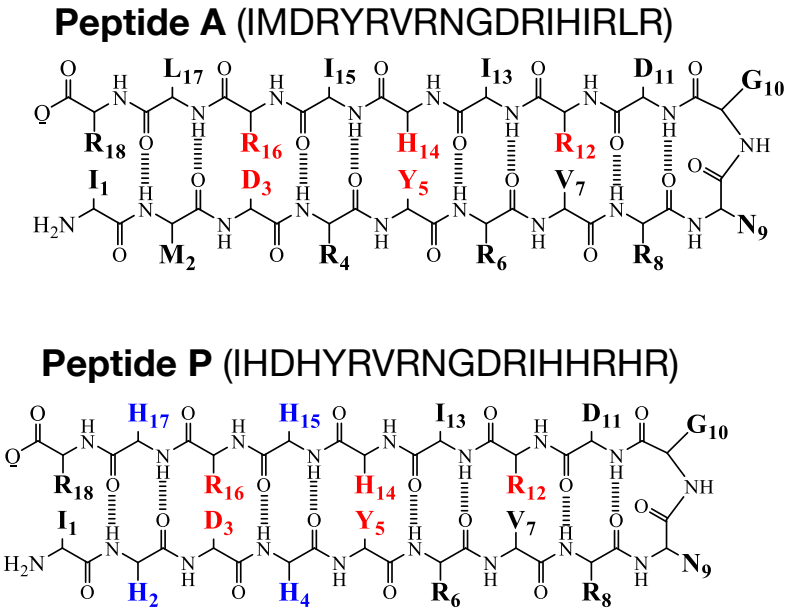

Figure 1: The sequence and secondary structure of the original peptide (Peptide A) and the peptide of interest for this study (Peptide P). The sequence also shows the cross-strand interactions between the residues.

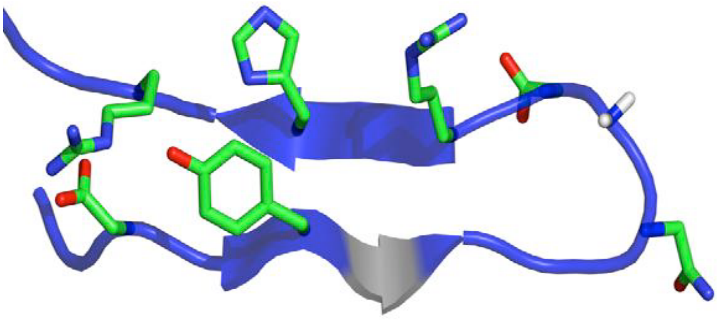

Figure 2: NMR tertiary structure of Peptide A (8).

The current study focuses on the metal-binding properties of Peptide P. Metals were introduced to the peptide and various spectroscopic methods such as circular

dichroism and UV-Resonance Raman Spectroscopy were implemented to determine if the metal is coordinating with the peptide. These metals will be manganese and zinc, based off of the OEC of Photosystem II and the zinc-histidine complex of carbonic anhydrase respectively (3, 11). Previous studies have shown that the Raman spectra of metal-bound histidine are different from metal-free histidine by the introduction of a band at 1287 cm^{-1}. Therefore if the introduced metal does bind to Peptide P a different spectrum should be observed when compared to that of Peptide P alone (10). If the metal does bind to Peptide P, then further studies will be conducted to study the properties of this metal-peptide complex. By developing a deeper understanding of Peptide P, more complex biomimetic metal-coordinating compounds can be created that may serve to inspire designs for more complex systems for commercial applications.

CHAPTER 3

MATERIALS AND METHODS

Peptide Engineering

A biomimetic peptides was engineered to contain four histidine residues that act as metal-binding site. Peptide P (IHDHYRVRNGDRIHHRHR) also allows the tyrosine and histidine residues to interact with each other through the hairpin shape (Figure 1). The peptide samples were synthesized by Genscript (Piscataway, New Jersey).

UV-Visible Spectroscopy

Several spectroscopic methods were used to characterize the interaction between the metal and Peptide P. UV-Visible spectroscopy was used to monitor how the tyrosine residue spectrum is affected by the introduction of the metal. The experiments will be conducted at both pD 5.5 and pD 7.5 on 100 μM solutions of peptide, to determine how the protonation state of the histidine residue affects coordination affinity. When measured at pD 7.5, the peptide was in 5 mM HEPES solution and when measured at pD 5.5, the peptide was in 5 mM acetate buffer. The samples were measured from 220 nm to 350 nm using a Shimadzu UV-Vis spectrophotometer. The parameters were as follows: scan speed, medium; sampling interval, 1nm; scan mode, single. Varying concentrations of metal, from 0.5 mM to 4 mM, were introduced to the peptide to create a titration curve. Two trials were averaged for data analysis.

Circular Dichroism Spectropolarimetry

Circular dichroism spectropolarimetry (CD) was conducted to see how the introduction of metal affects the secondary structure of the peptide. Spectra were obtained using 100 μM solutions of peptide 5 mM HEPES solution on a JASCO J-810 CD Polarimeter. The parameters were as follows: sensitivity, 100 mdeg; data pitch, 1 nm; scan speed, 50 nm/min; bandwidth, 1 nm; accumulations, 8. Samples were measured from 180 nm to 250 nm at 20 °C and then heated to 80 °C to see the thermodynamic stability of Peptide P both with and without the metal. The samples will then be cooled to 20 °C to determine if the folding is reversible. Two trials were averaged for data analysis.

UV-Resonance Raman Spectroscopy

UV-Resonance Raman (UVRR) spectroscopy of 1 mM peptide samples in 5mM HEPES solution was also used to monitor shifts in the histidine bands at room temperature. Histidine has a distinct Raman spectrum that can be easily monitored using a 244 nm monochromatic laser. To prevent damage to the sample, 2 mL of sample was cycled through using a flow cell pump. The spectral resolution was 6 cm^{-1}. A total of 4 accumulations were collected with 64 second intervals per spectra acquisition. If the metals do interact with the peptide, a new band may be visible.

CHAPTER 4

RESULTS

UV-Visible Spectroscopy

UV-Visible spectroscopy was employed to measure how the presence of metal alters the tyrosine absorbance spectrum. Because the pKa of histidine is 6.0, measurements of Peptide P at both pD 5.5 and pD 7.5 were conducted to determine how the protonation state of histidine affects the peptide's metal coordination affinity. A zinc metal titration was performed at both pDs, meaning that the peptide was exposed to zinc at different concentrations to see if there is a distinct shift in the tyrosine absorbance band as the peptide is exposed to more metal. Figure 3 shows the results of the study. At pD 5.5, no visible shift in the spectra can be seen at any concentration ratio of metal to zinc (Figure 3.B). In fact, there seems to be complete overlap between the spectra, with a peak around 273 nm. However, at pD 7.5 there is a visible shift in the spectra as the metal is introduced to the peptide environment (Figure 3.A). In fact as the concentration of metal increases, the shift from the control spectra (the black trace) is more prominent. The shift from the 1:8 peptide to metal solution (green trace) is the most noticeable, with its peak being at 277 nm.

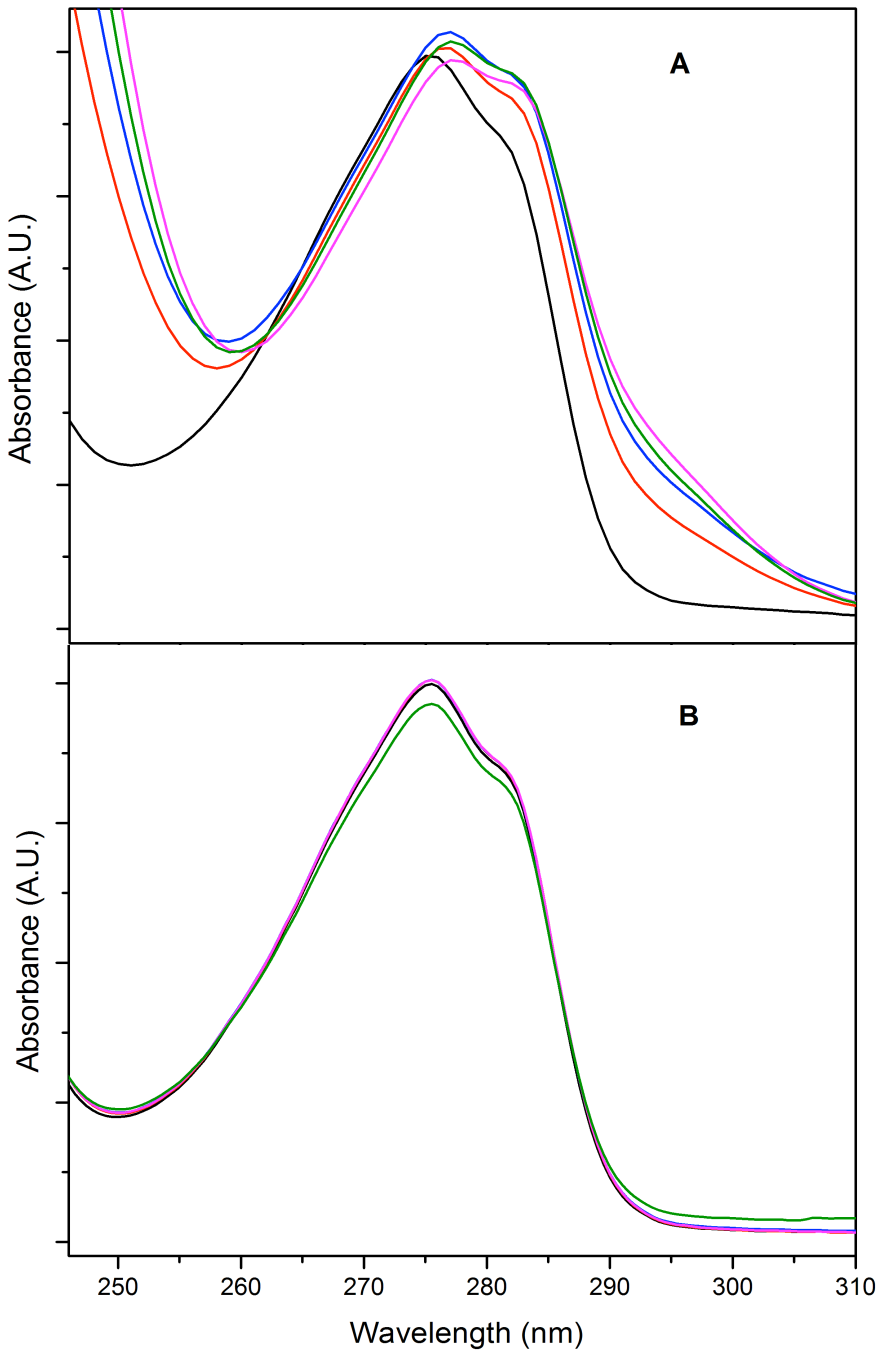

Figure 3: Absorption spectra of 0.5 mM Peptide P at pD 7.5 (A) and at pD 5.5 (B) with various concentrations of zinc: 0 mM (black), 0.5 mM (red), 1 mM (blue), 2 mM (pink), and 4 mM (green). Each tick marks denotes 0.1 absorbance unit. At pD 7.5 the buffer was 5 mM HEPES; at pD 5.5 the buffer was 5 mM acetate. The figure shows an average of two trials.

UV-Resonance Raman Spectroscopy

The UVRR spectra collected was in order to observe differences between the spectra of the Peptide P with and without zinc. There are a few characteristic peaks evident in the spectra such as the D_2O peak at 1200 cm^{-1}, and the imidazole band around 1565 cm^{-1} (Figure 4). However there are a few distinctions between the UVRR characterization of Peptide P with and without zinc. For one, with zinc the intensity of the spectra is increased. Figure 4 also shows a 1336 cm^{-1} peak of the Peptide P spectrum is shifted in the Peptide P + Zn spectrum. There is also a band at 1287 cm^{-1} present in the Peptide P + Zn spectrum, not in the control. An extra peak can also be observed in the blue trace at 1175 cm^{-1}, which slightly overlaps with the D_2O peak around the same area. It should also be noted that the 1565 cm^{-1} peak of Peptide P becomes a shoulder when zinc is introduced to the peptide environment.

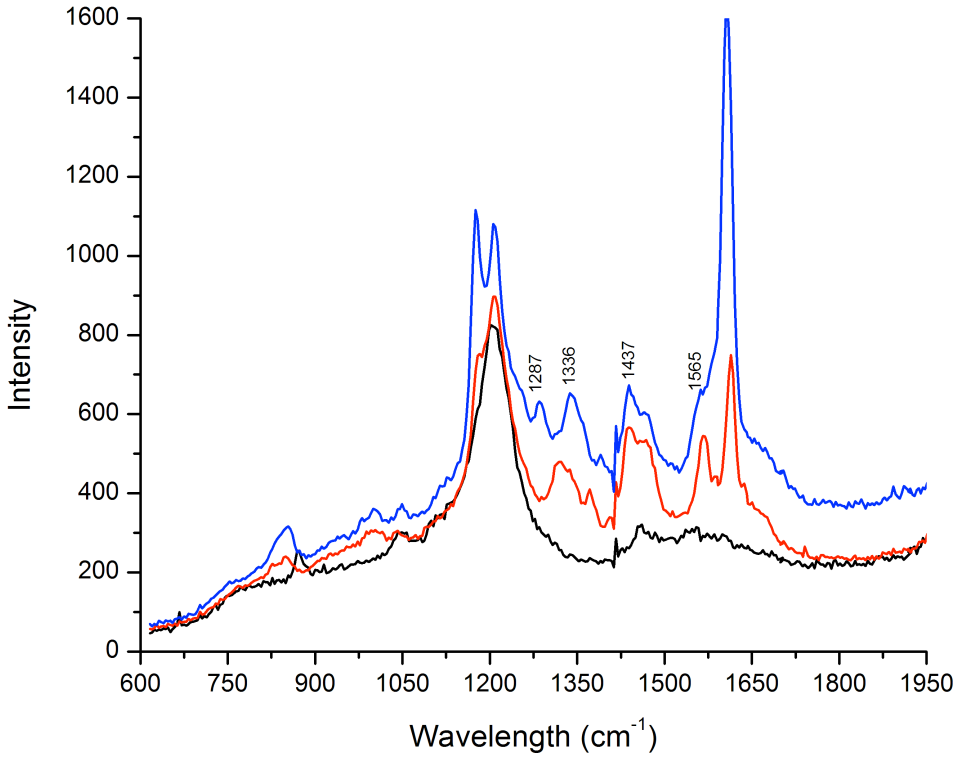

Figure 4: UVRR spectra of 5 mM HEPES buffer (back trace), 1 mM Peptide P by itself (red trace) and with 1 mM zinc (blue trace) all taken at pD 7.5. The data was collected using a 244 nm laser with 3 mW power. Each tick marks denote 100 units of intensity. This data is from one representative trial.

Circular Dichroism

The CD spectrum of Peptide P shows the characteristic peak of a beta-hairpin at 198 nm (Figure 5). This peak was also identified in Peptide A, and was confirmed to be a beta- hairpin structure after the NMR analysis of Peptide A was conducted (Figure 2). The 80 °C melt shows that the peptide is able to denature under heat, however as seen by the 20 °C post-heating spectra (blue trace) this unfolding is reversible, and the original 20 °C is almost fully obtained. The 80 °C melt also shows a shift in the negative peak to 200 nm.

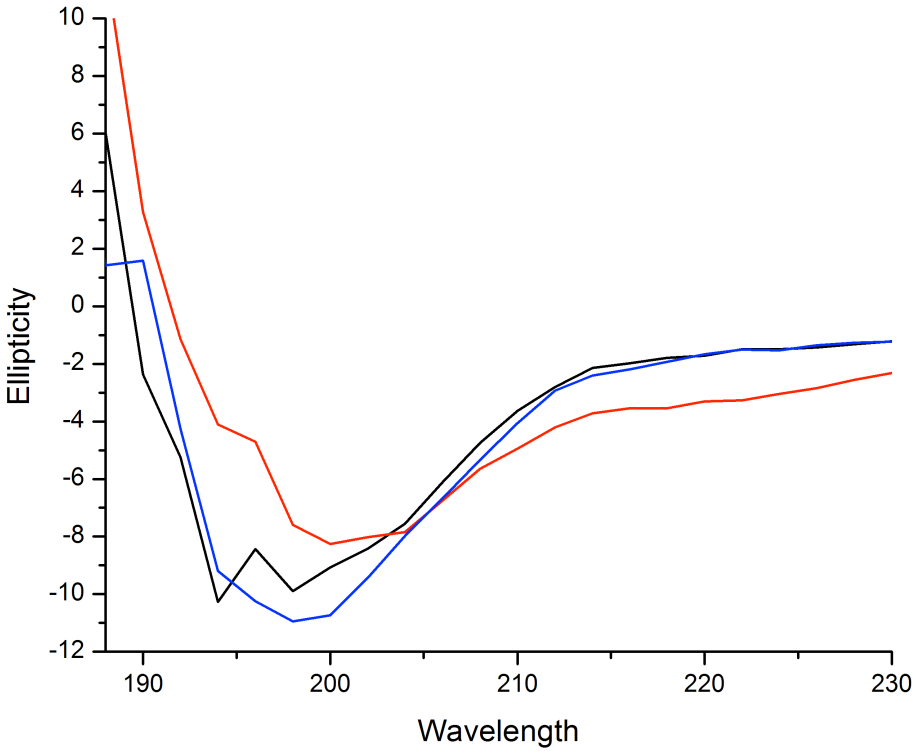

Figure 5: CD spectra of 100 μM Peptide P at pH 7.5. The spectra was collected at 20°C (black trace), 80°C (red trace), and again at 20°C (blue trace). Each tick mark denotes 1 mdeg. This figure is an average of two separate trials.

The CD spectra of Peptide P with zinc also showed the characteristic peak of a beta-hairpin at 198 nm (Figure 6). However, there is a broad peak from 210 nm to 220 nm in all three of the spectra. The ellipticity of the spectra does not waver with temperature either, which is unlike what was observed in the CD spectra of Peptide P alone (Figure 5). The 80 °C melt (red trace) also shows that the negative peak has shifted to 200 nm.

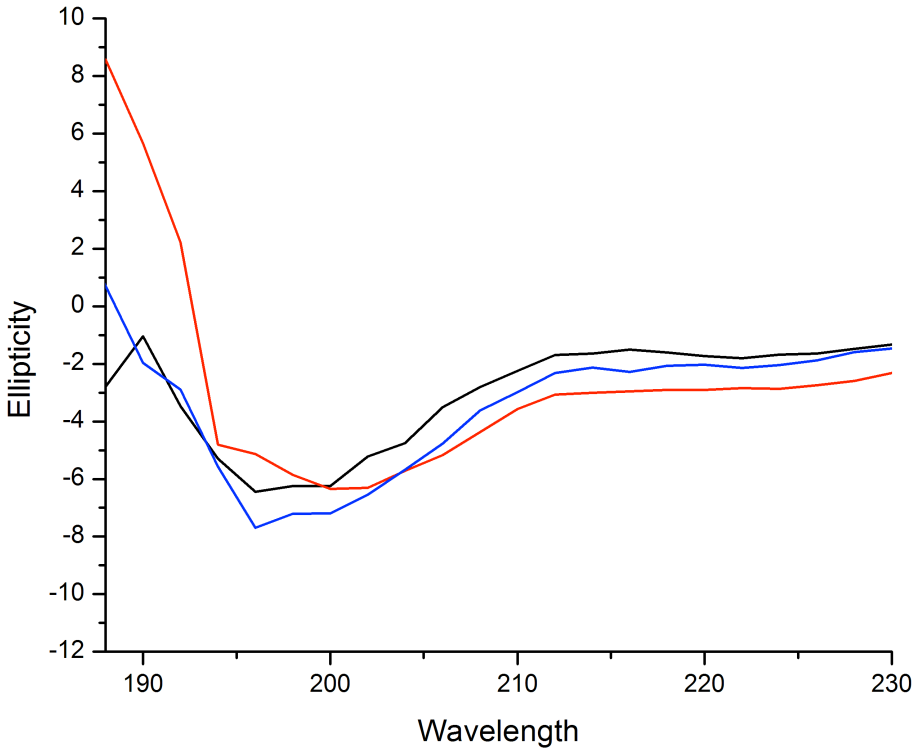

Figure 6: CD spectra of 100 µM Peptide P with 100 µM zinc at pH 7.5. The spectra was collected at 20°C (black trace), 80°C (red trace), and again at 20°C (blue trace). Each tick mark denotes 1 mdeg. This figure is an average of two separate trials.

CHAPTER 5

DISCUSSION

Based on the UV-Vis data, it can be concluded that the zinc metal is coordinating with Peptide P, due to the deprotonated state of the histidine. The gradual shifts in the metal titration spectra in Figure 3A indicate a shift in the electronic distribution of the tyrosine residue. A plausible cause for this is that the metal is coordinating to Peptide P Conversely, in Figure 3B at pD 5.5, when the histidine residues of the peptide are protonated, there is no shift in the spectra when the zinc is introduced to the peptide. This indicates that the metal is not binding to the peptide. Overall, the evidence from the UV-Vis spectra point to the conclusion that zinc is indeed coordinating with Peptide P.

The UVRR data (Figure 4) further strengthens this notion. Previous studies have characterized the Raman spectra of metal-bound imidazole complexes (10). Notably, the 1287 cm^{-1} band appears when a metal is bound to the histidine imidazole of Cu/Zn superoxide dismutase, which is found in the current study's results as well (10). Alongside this 1287 cm^{-1} band appearance, the shifted band at 1336 cm^{-1} and the higher intensity of the 1602 cm^{-1} tyrosine ring stretch band occur when the zinc is coordinated with the peptide.

The beta-hairpin secondary structure of Peptide P was confirmed through the CD studies (Figure 5). This conclusion is based on previous studies of Peptide A, where a similar CD spectrum was obtained and the resulting NMR spectra confirmed the beta-hairpin shape (8). However, the characteristic negative peak of beta-hairpins tends to be near 210 nm. The results do not show any characteristics that are similar to other secondary structures such as an alpha helix or random coils.

Interestingly, when zinc was introduced to Peptide P the resulting spectra changed (Figure 6). Although the characteristic negative peak is there, the ellipticity of the peak in Figure 6 is less prominent than that of Peptide P alone. Also, the broad peak from 210 nm to 220 nm is found neither in the Peptide P nor in a characteristic beta hairpin CD spectrum. Because this peak is consistent over several trials and the overall spectral shape is retained after heating the sample, the secondary structure of Peptide P may not be random coils. The stability of Peptide P when heated is also a notable characteristic of the peptide. This interpretation is based off of preliminary data, and additional experiments will be conducted to average out the data. With these different pieces of evidence in mind, it may be possible that Peptide P is forming amyloid fibers, with the zinc metal serving as a link between different peptides. This idea of amyloid fiber formation accounts for the shape of the CD spectra as well as the conferred stability of Peptide P when zinc is introduced.

CHAPTER 6

CONCLUSIONS

This study looked at the ability of a biologically inspired designed peptide (Peptide P) to bind to a metal cofactor. Through various spectroscopic methods, the peptide was characterized and furthermore found to bind to metal. For this study zinc was used as the metal cofactor, which is also a common metal used in biological metalloproteins such as carbonic anhydrase. The UV-Vis, UVRR, and CD spectra all point to the indication that zinc does bind to the peptide. The concentration of peptide to zinc binding also seems to consistently be one to one. This zinc could further be utilized to form stable amyloid fibers of Peptide P.

Further studies can be done to distinguish if the peptide is actually forming amyloid fibers as the CD spectra indicate using electron microscopy. Also, electron paramagnetic resonance studies could be conducted in order to characterize the electronic properties of the metal-bound peptide. Overall, Peptide P can serve as a usable model to further study and understand the properties of metalloproteins.

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